Remarks

Amendments to the claims

Claims 1, 6, 8, 9, 11, 13-18, 23-24 and 26-27 have been amended. Support is found at least in the original claims and at p. 10, line 18 to p. 16, line 10.

Rejections under 35 U.S.C. §112, first paragraph

Claims 1-27 and 31-33 were rejected under 35 U.S.C. §112, first paragraph, for allegedly lacking sufficient description and enablement. The rejections are respectfully traversed if applied to the amended claims.

The Examiner stated in the Office Action mailed October 11, 2000, that the applicants provided bacterial genes encoding PHA synthase from *Clostridium acetobutylicum* and a single gene encoding D-specific enoyl-CoA hydratase from *Aeromonas caviae* but no other non-bacterial genes encoding PHA synthase (Office Action, p. 4, first paragraph). Specifically, the Examiner stated that the applicants describe "bacterial genes encoding each of PHB polymerase, PHA polymerase, β-ketothiolase, and β-ketoacyl-CoA reductase." The Examiner further stated that the applicants teach a single gene encoding each of crotanase, butyryl-CoA dehydrogenase, and 3-hydroxybutyryl-CoA dehydrogenase from *Clostridium acetobutylicum* and a single gene encoding D-specific enoyl-CoA hydratase from *Aeromonas caviae* (Id.). The Examiner further asserted that because non-bacterial genes encoding the listed enzymes were not disclosed, the specification allegedly lacks sufficient description. The Examiner maintained that University of California v. Eli Lilly and Co., 43 USPQ2d 1398 (Fed. Cir. 1997) ("Eli Lilly") supports the position that the all the genes encompassed in the claims should be specifically listed in the

specification.

The applicants respectfully disagree. First, the claims are limited to bacterial genes. This moots most of the Examiner's basis for rejection.

First, the standard for determining compliance with the written description requirement is whether the description clearly allow persons of ordinary skill in the art to recognize that the applicant invented what is claimed (see In re Gosteli, 872 F.2d 1008, 1012, 10 U.S.P.Q.2d 1614, 1618 (Fed. Cir. 1991). Omission of what is generally known in the art does not render the description insufficient (see MPEP § 2163.02, second paragraph (stating that the subject matter of the claim need not be described literally)). In Eli Lilly, the issue was whether an adequate description of rat insulin cDNA is an adequate description of the broad classes of vertebrate or mammalian insulin cDNA. Eli Lilly, 43 USPQ2d at 23. Here, however, the issue is whether the disclosure description is adequate when the claimed subject matter uses **known genes** to generate a transgenic organism with a construct formed of the **known genes** that are integrated into the chromosome of the organism. With the teachings and guidance provided in the specification, one of ordinary skill in the art is able and use the bacterial transgenes defined by the claims.

The critical aspect of the claimed subject matter is the supply of R-3-hydroxyhexanoyl CoA in the organism. The specification describes, for example, butyrate fermentation pathway (p. 12, line 18 to p. 14, line 2; Figure 3), fatty acid oxidation pathway (p. 14, line 5 to p. 15, line 13; Figure 4), and fatty acid biosynthetic pathway (p. 15, lines 5-23; Figure 5). In the butyrate fermentation pathway, the applicants identified a thiolase and a reductase specific for 3-

ketohexanoyl CoA and a PHB polymerase that accepts both 3-hydroxybutyryl CoA and 3-hydroxyhexanoyl CoA (p. 12, lines 23 and 26-28). The specification provides guidance to obtain thiolase genes (p. 13, line 25-26) with *fadA* and *atoB* listed as examples (p. 13, line 28); and suitable reductase and polymerase genes (p. 14, lines 1-2). In the fatty acid oxidation pathway, the specification discloses that the combined action of an epimerase and a PHA polymerase provides C6-C14 monomers for PHA (p. 14, lines 10-14; Figure 5) with the epimerase being the key element. A suitable epimerase is encoded, for example, by *FaoAB* (p. 14, lines 21-26). The specification further provides guidance for obtaining *FaoAB* from other bacteria (p. 14, line 30 to p. 15, line 3). In the fatty acid biosynthetic pathway, the specification discloses that the critical enzymatic activity is the conversion of the 3-hydroxyacyl ACP to the CoA derivative which can be accomplished by thioesterases and acyl CoA synthases or acyl ACP:CoA transferases (p. 15, lines 18-20). The specification further provides that the genes encoding these enzymes can be identified and isolated from *P. putida* in screening procedures (p. 15, lines 14-15). Identification and isolation of genes encoding thioesterases and acyl CoA synthases can be readily carried out by one of ordinary skill in the art using knowledge available in the art. For example, Voelker et al. reported the isolation and characterization of *FatB* cDNA encoding FatB ACP thioesterases in 1997 (Voelker et al., "Broad-range and binary-range acyl-acyl-carrier protein thioesterases suggest an alternative mechanism for medium-chain production in seeds" in *Plant Physiol.* 114(2):669-77 (1997)). Naggert et al., described the isolation and characterization of *TesB* encoding *E. coli* thioesterase II (Naggert et al., "Cloning, sequencing, and characterization of *E. coli* thioesterase II" in *J. Biol. Chem.* 266(17):11044-50 (19919)).

The specification provides sufficient description to one of ordinary skill in the art. In addition to the specific teachings provided in the specification, genes encoding PHA synthase are well known in the art (see, for example, Tombolini et al.; "Poly-beta-hydroxybutyrate (PHB) biosynthetic genes in Rhizobium meliloti 41," in *Microbiology* 141(Pt 10):2553-9 (1995); Hustede et al., "Cloning of poly(3-hydroxybutyric acid) synthase genes of Rhodobacter sphaeroides and Rhodospirillum rubrum and heterologous expression in Alcaligenes eutrophus," in FEMS Microbiol Lett. 72(3):285-90 (1992); Poirier et al., "Synthesis of high-molecular-weight poly([R])-(-)3-hydroxybutyrate) in transgenic Arabidopsis thaliana plant cells," in *Int. I. Biol. Macromol.* 17(1):7-12 (1995); Nawrath et al., "Targeting of the polyhydroxybutyrate biosynthetic pathway to the plastids of Arabidopsis thaliana results in high levels of polymer accumulation," in *Proc. Natl Acad. Sci. USA* 91(26):12760-4 (1994)).

The Examiner also rejected all the claims for allegedly lacking enablement. Specifically, the Examiner alleged that the enabled subject matter is not in commensurate with the scope of the claims. The applicants respectfully disagree. First, the specification details, the material and methods for isolating, amplifying and cloning genes (p. 18, line 15 to p. 19, line 10; Example 1). Specifically, the specification teaches genes encoding PHA synthase enzymes such as those from *N. salmonicolor*, *A. caviae*, *C. testosteroni*, *T. pfenigii*, *P. denitrificans*, *natans*, *Z. ramigera*, *acetobutylicum*, *R. eutroph*, *P. putida*, and *V. fischeri* (p. 19, line 11 to p. 26, line 12, Examples 1-6). Second, even if one argued that the disclosed methods do not cover isolating, amplifying and cloning all the genes encompassed by the claims, the art is replete with the documentation of isolating, amplifying and cloning genes encoding enzymes from various sources which are

involved in the PHA synthesis pathways (see, for example, Liebergesell et al., "Cloning and nucleotide sequences of genes relevant for biosynthesis of poly(3-hydroxybutyric acid) in Chromoatium vinosum strain D," in *Eur. J. Biochem.* 209(1):135-50 (1992); Tombolini et al., "Poly-beta-hydroxybutyrate (PHB) biosynthetic genes in Rhizobium meliloti 41," in *Microbiology* 141(Pt 10):2553-9 (1995); Willis et al., "The phbC (poly-beta-hydroxybutyrate synthase) gene of Rhizobium (Sinorhizobium) meliloti and characterization of phbC mutants," in *Can J. Microbiol.* 44(6):554-64 (1998); Cevallos et al., "Genetic and physiological characterization of a Rhizobium etli mutant strain unable to synthesize poly-beta-hydroxybutyrate," in *J. Bacteriol.* 178(6):1646-54 (1996); Mandon et al., "Poly-beta-hydroxybutyrate turnover in Azorhizobium caulinodans is required for growth and affects nifA expression," in *J. Bacteriol.* 180(19):5070-6 (1998); and Maehara et al., "Analyses of a polyhydroxyalkanoic acid granule-asociated 16-kilodalton protein and its putative regulator in the pha locus of Paracoccus denitrificans," in *J. Bacteriol.* 181(9):2914-21 (1999)). Contrary to the Examiner's assertion that one of ordinary skill in the art would need to **identify** those genes which are functionally related to the genes specifically disclosed as examples in the specification, there is no such requirement. One of ordinary skill in the art can readily use the publically available knowledge relating to methods to isolate, amplify and clone genes which have been documented to be involved in the PHA synthesis pathways.

The Examiner further asserted that the successful isolation of genes as taught in the art does not enable isolation of the claimed genes and transgenic organisms having the genes. First, the claims do not require any particular genes other that those known in the art. The claimed

subject matter is a method of making PHAs containing 3-hydroxyhexanoate by growing a transgenic organism generated by integrating into its chromosome a bacterial transgene encoding a **known** PHA polymerase incorporating C6 substrates or a known D-specific enoyl-CoA hydratase. Second, the specification provides sufficient guidance for one of ordinary skill in the art to obtain and use known genes (p. 10, line 18 to p.15, line 23; Examples 1-6). Third, one of ordinary skill in the art would be able to isolate and amplify genes encoding PHA synthase enzymes, as described.

The Examiner stated that it is not clear that any other bacteria produce the claimed genes. The applicants draw the Examiner's attention to the fact that biological PHAs production occurs only if enzymes involved in the PHA synthesis pathways which are encoded by genes are present. Therefore, logically, if an organism produces C5-C15 PHAs, the PHA synthase enzymes, and thus the genes encoding the enzymes, must be present. Bacteria which produce C5-C15 PHAs are well known in the art (see, for example, Kraak et al., "Polymerase C1 levels and poly(R-3-hydroxyalkanoate) synthesis in wild-type and recombinant *Pseudomonas* strains" in *J. Bacteriol.* 179(16):4985-4991 (1997) ("Kraak"); Kranz, et al., "Positive selection systems for discovery of novel polyester biosynthesis genes based on fatty acid detoxification" in *Appl. Environ. Microbiol.* 63(8):3010-3 (1997) ("Kranz"); and Witholt, "Perspectives of medium chain length poly(hydroxyalkanoates), a versatile set of bacterial bioplastics" in *Curr. Opin. Biotechnol.* 10(3):279-85 (1999) ("Witholt")).

The Examiner further stated there is no definitive evidence that other bacteria produce genes that encode a PHA polymerase that incorporates C6 substrates or a D-specific enoyl-CoA

hydratase. First, by definition, PHA polymerase is the enzyme that catalyzes PHA production. One of ordinary skill in the art of polymer chemistry would appreciate that, in order for the enzyme, the PHA polymerase, to catalyze hydroxyalkanoate polymerization, the enzyme must incorporate the substrate to initiate the polymerization reaction. Therefore, the formation of PHAs containing hydroxyhexanoate is conclusive evidence that the polymerase incorporates C6 substrate during the course of polymerization. As the cited examples, Kraak, Kranz, and Withold, all show, formation of PHAs having C6 monomers is well documented. The polymerase catalyzing the formation of PHAs in Kraak, Kranz, and Withold, must have incorporated C6 substrates. The applicants particularly direct the Examiner to Tables 2 and 3 of Withold which reported a polymerase incorporating C6 substrates (p. 4988 and 4989, Tables 2 and 3).

The claimed subject matter uses D-specific enoyl-CoA hydratase to hydrolyze enoyl-CoA to form R-3-hydroxyhexanoyl CoA (p. 23, line 29-29) which is the proper substrate to form PHAs containing R-3-hydroxyhexanoate. The specification clearly teaches that an *phaJ* gene encoding an enoyl-CoA hydratase (p. 24, lines 4-6). Further, D-specific hydratases, and the genes encoding the same, are well known in the art and can be found in many other species. For example, Caira et al., "Differential regulation by a peroxisome proliferator of the different multifunctional proteins in guinea pig: cDNA cloning of the guinea pig D-specific multifunctional protein 2" in *Biochem. J.* 330(pt 3):1361-8 (1998), describes a D-specific multifunctional protein 2 which catalyzes the hydration of 2-enoyl-CoA in D-specific fashion. D-specific enoyl-CoA hydratase is also reported in Dieuaide-Noubbani et al., "Identification and

characterization of the 2-enyol-CoA hydratases involved in peroxisomal beta-oxidation in rat liver" in *Biochem. J.* 321(Pt 1):253-9 (1997).

The Examiner further stated that although bacterial genes encoding PHA synthase enzymes can be expressed in other bacteria or plants, the phenotypic effects of the introduction of transgenes is unpredictable. Contrary to the Examiner's assertion, as shown by the references cited above, the art of using transgenic organisms to produce PHAs is predictable (see Kraak, – Kranz, and Witholt, supra). Further, medium-chain-length PHAs formed of substrates including C5-C16 substrates have been successfully produced by transgenic plants (see Witholt, supra; see also Poirer, "Production of polyesters in transgenic plants" in *Adv. Biochem. Eng. Biotechnol.* 71:209-40 (2001) (Review) in which the author unequivocally stated that a range of medium-chain-length PHAs has been produced in transgenic plants).

The Examiner's statement that the production of PHAs require three genes, PHB polymerase, beta-ketothiolase and acetoacetyl-CoA reductase is well taken. This is not accurate in all cases, since substate can in some cases be provided by alternative pathways and only the polymerase is absolutely essentially. However, the claims, as amended, requires the organism to produce PHA, therefore inherently requiring PHB polymerase, beta-ketothiolase and acetoacetyl-CoA reductase.

In sum, the claimed subject matter is fully described and enabled to one of ordinary skill in the art.

Rejections under 35 U.S.C. §112, second paragraph

Claims 1-27 and 31-33 were rejected under 35 U.S.C. § 112, as indefinite. The claims

have been amended in view of the applicants appreciate the Examiners insightful comments in this regard.

Rejection under 35 U.S.C. §102

Claims 1, 2, 5-11, 15, 31, and 32 were rejected under 35 U.S.C. §102(a) as disclosed by Fukui, et al., J. Bacteriol. 179:4821-4830 (1997). This rejection is respectfully traversed.

Fukui discloses production of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) in bacteria by expression of plasmids in the bacteria. The claims require a transgene encoding either a PHA polymerase incorporating C_6 substrates or D-specific enoyl-CoA hydratase be incorporated into the genome of a plant or bacterium. As the Examiner acknowledges, Fukui does not disclose integration of isolated genes. Therefore Fukui does not disclose the claimed subject matter.

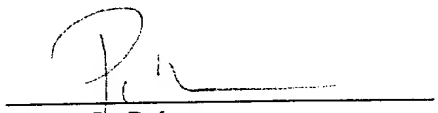
The Examiner, however, held that it was well known in the art that bacterial transformation resulted in incorporation of the introduced DNA into the genome. This statement is unwarranted. Bacterial transformation does not result in incorporation of the transgenes into the genome. In fact, as one of ordinary skill in the art would appreciate, expression of the genes encoding PHA synthase in plasmids for PHA production generally requires high copy number of plasmids. In addition, the expression of the genes are highly unstable. Expression of the genes in plasmids is different from incorporation of the genes into the chromosome.

Fukui also does not make obvious the claims under 35 U.S.C. § 103. Fukui does not provide the motivation for one of ordinary skill in the art to modify Fukui to the claimed subject matter as applicants have done. Secondly, the claimed method does not require high copy

number of plasmids nor measures to stabilize plasmids. Therefore, one of ordinary skill in the art, taught by Fukui, would not have had not have a reasonable expectation of success of the claimed method. Therefore, the claimed subject matter is not obvious over Fukui under 35 U.S.C § 103. Even if one argued the claims are *prima facie* obvious over Fukui, the applicants have obtained unexpected results to rebut the *prima facie* obviousness, if any. As discussed above, one of ordinary skill could not have reasonably expected the claimed method to be successful because the method does not require a high copy number of the plasmids and stabilization of the plasmids. Yet, the results obtained by the applicants are at least comparable to that disclosed by Fukui.

Allowance of all of claims 1-27 and 31-33 as amended is earnestly solicited. All claims as now pending are attached in an appendix to facilitate the examiner's review.

Respectfully submitted,



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Appendix I: Claims as pending upon entry of amendment

1. (twice amended) A method for the biological production of polyhydroxyalkanoate containing 3-hydroxyhexanoate comprising growing a transgenic organism selected from the group consisting of a transgenic bacterium and a transgenic plant having at least one bacterial transgene encoding an enzyme selected from the group consisting of a PHA polymerase incorporating C_6 substrates and a D-specific enoyl-CoA hydratase, integrated into the chromosome, [under conditions suitable for] wherein production of polyhydroxybutyrate-polyhydroxyvalerate containing 3-hydroxyhexanoate by the transgenic organism occurs.

2. (amended) The method of claim 1 wherein the organism is a plant.

3. (amended) The method of claim 2 wherein the organism is a plant selected from the group consisting of an oil crop plant and a starch accumulating plant.

4. (amended) The method of claim 3 wherein the plant is selected from the group consisting of *Brassica*, sunflower, soybean, corn, safflower, flax, palm, coconut, potato, tapioca, cassava, alfalfa, grass, and tobacco.

5. (amended) The method of claim 1 wherein the organism is a bacterium selected from the group consisting of *Escherichia, Klebsiella, Ralstonia, Alcaligenes, Pseudomonas,* and *Azotobacter*.

6. (amended) The method of claim 1 wherein the [organism is genetically engineered to express or overexpress] transgene encodes a PHA polymerase incorporating C_6 substrates.

7. (amended) The method of claim 6 wherein the polymerase is from *Aeromonas caviae, Comamonas testosteroni, Thiocapsia pfenigii, Chromatium vinosum, Bacillus cereus, Nocardia*

carolina, Nocardia salmonicolor, Rhodococcus ruber, Rhodcoccus rhodocrous, and *Rhodospirilum rubrum.*

8. (twice amended) The method of claim 1 wherein the organism [is genetically engineered to redirect] directs metabolites to production of 3-hydroxyhexanoyl-CoA.

9. (amended) The method of claim 8 wherein the [organism is genetically engineered using] transgene encodes a D-specific enoyl-CoA hydratase gene.

10. (amended) The method of 9 wherein the hydratase gene is isolated from a bacterium selected from the group consisting of *R. eutropha, Klebsiella aerogenes, P. putida,* and *Aeromonas caviae.*

11. (twice amended) The method of claim 8 wherein the organism [is genetically engineered using] has the genes encoding the enzymes in a butyrate fermentation pathway.

12. (amended) The method of claim 11 wherein the enzymes in the butyrate fermentation pathway are from *Clostridium acetobutylicium* or *Thermoanaerobacterium thermosaccharolyticum.*

13. (twice amended) The method of claim 11 wherein the organism [is genetically engineered to convert] converts butyrate to butyryl CoA or butyryl CoA to crotonyl CoA.

14. (twice amended) The method of claim 11 wherein the organism [is genetically engineered to express] expresses a broad range reductase that is active on C_6 substrates.

15. (twice amended) The method of claim 11 wherein the organism [is genetically engineered to express] expresses a polymerase that accepts 3-hydroxyhexanoyl CoA.

16. (twice amended) The method of claim 11 wherein the organism [is genetically engineered to express] expresses a thiolase accepting acetoacetyl CoA.

17. (twice amended) The method of claim 11 wherein the organism [is genetically engineered to express] expresses an enzyme selected from the group consisting of thiolases specific for 3-ketohexanoyl CoA, reductase active on 3-ketohexanoyl CoA, and 3-hydroxyhexanoyl CoA.

18. (twice amended) The method of claim 8 wherein the organism [is further genetically engineered to express] expresses one or more fatty acid biosynthetic enzymes.

19. The method of claim 18 wherein the fatty acid biosynthetic enzymes are enzymes converting acyl ACP to acyl CoA.

20. The method of claim 19 where the enzymes are selected from the group consisting of ACP-CoA transacylase, acyl ACP thioesterase, and acyl CoA synthase.

21. The method of claim 20 wherein the enzymes are acyl ACP thioesterase and acyl CoA synthase.

22. (amended) The method of claim 18 wherein the enzymes are from *E. coli*.

23. (twice amended) The method of claim 8 wherein the organism [is further genetically engineered to express] expresses one or more enzymes forming a fatty acid oxidation complex.

24. (amended) The method of claim 23 wherein the [fatty acid oxidation complex comprises enzymes] one or more enzymes are selected from the group consisting of enzymes epimerizing S-3 hydroxyhexanoyl CoA and enzymes reducing 3-ketohexanoyl CoA.

25. (amended) The method of claim 24 wherein the enzymes are from *Nocardia salmonicolor*.

26. (twice amended) The method of claim 24 wherein the [epimerizing] enzymes epimerizing S-3 hydroxyhexanoyl CoA are from the *Pseudomonas putida* FaoAB complex.

27. (amended) The method of claim 23 wherein the organism [that is genetically engineered] accumulates 3-ketohexanoyl CoA due to a lack of a thiolase.

31. (twice amended) A transgenic bacterium or plant for use in any of the methods of claims [1-30] 1-27.

32. (amended) The transgenic bacterium of claim 31.

33. (amended) The transgenic plant of claim 31 wherein the plant is a higher order plant.